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RESEARCH ARTICLE

In Vitro Antioxidant and Free Radical Scavenging Activity of *Alstonia scholaris* Linn. R.Br.

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ABSTRACT

The present work is carried out to study the antioxidant potential of ethanolic extract of *Alstonia scholaris* Linn. (Apocynaceae) using various in vitro tests including 1, 1-diphenyl-2-picryl-hydrazil ($DPPH^{\cdot}$) free radical scavenging, metal ion chelating, hydrogen peroxide scavenging, superoxide anion radical scavenging, and ferric thiocyanate reducing ability. Ethanolic extract of *Alstonia scholaris* had significant ($p<0.01$) ($DPPH^{\cdot}$) free radical scavenging (63%), metal ion chelating (74.88%), hydrogen peroxide scavenging (72.28%), superoxide anion radical scavenging (67.66%) and significant ($p<0.05$) ferric thiocyanate reducing activities. These various antioxidant activities were compared to standard antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxy toluene (BHT), l- ascorbic acid and α-tocopherol. The results indicated that ethanolic extract of *Alstonia scholaris* Linn. possess antioxidant property. The results observed were comparable to antioxidant properties of BHA, BHT, l-ascorbic acid and α-tocopherol.

Keywords: *Alstonia scholaris, Antioxidant, Radical Scavenging, Lipid Peroxidation*

Alstonia scholaris Linn. is an antimalarial [1] drug used in the marketed Ayurveda preparation Ayush-64, NRDC, India. The plant *Alstonia scholaris* Linn. R.Br., belongs to the family Apocynaceae and is native to India. It grows throughout India, in deciduous and evergreen forests and also in plains [2].

The bark is bitter, astringent, acrid, thermogenic, digestive, laxative, anthelmintic, febrifuge, antipyretic, depurative, galactogogue, stomachic, cardiotonic and tonic [2]. It is useful in fevers, malarial fevers, abdominal disorders, dyspepsia, leprosy, skin diseases, pruritus, tumours, chronic and foul ulcers, asthma, bronchitis, cardiopathy, helminthiasis, agalactia and debility [2,3]. In folk medicine, milky juice is applied on wounds, ulcers and rheumatic pains; mixed with oil and dropped into ear, it relieves ear ache. Juice of the leaves and tincture of the bark acts in certain cases as a powerful galactogogue. The drug is also used in cases of snake-bite [2].

The methanolic extract of this plant was found to exhibit pronounced antiplasmodial activity [4]. The plant is reported to have anti-mutagenic effect [5]. The bark extract of *Alstonia scholaris* has immunostimulating effect. The aqueous extract at low dose induced the cellular immune response while at high dose inhibited the delayed type of hypersensitivity reaction [6]. Echitamine chloride, an indole alkaloid, extracted from the bark of *Alstonia scholaris* has promising anticancer [7,8] effect against sarcoma. The plant has hepatoprotective activity on liver injury induced by CCl_4, β-D-galactosamine, acetaminophen and ethanol [9]. Recently we reported the wound healing and anti-inflammatory activities of *Alstonia scholaris* Linn [10]. The plant *Alstonia scholaris* Linn. is reported to possess *in vitro* nitric oxide scavenging activity in preliminary studies [11]. The nitric oxide scavenging property of a compound is evidence for free radical and ROS scavenging properties [12]. Several studies have demonstrated that plants produce potent antioxidants and represent important sources of natural antioxidants [13-15]. As there is paucity for the *in vitro* antioxidant activity of *Alstonia scholaris* Linn, the present study was taken to explore the *in vitro* antioxidant status of ethanolic extract of *Alstonia scholaris* Linn using various *in vitro* antioxidant tests.

MATERIALS AND METHOD

Plant Material and Extraction

The leaves of *Alstonia scholaris* (Family: Apocynaceae) were collected in the month of April May 2006

from hills of Savanthwadi, Maharashtra, India. The plant material was taxonomically identified by the Botanical Survey of India (BSI), Pune and a voucher specimen AS-1 was retained in herbarium of BSI, Pune for future reference. The dried powdered leaves (500 g) were defatted using petroleum ether and subjected to subsequent extraction in a Soxhlet apparatus by using chloroform and ethanol. The solvents were removed from the respective extracts under reduced pressure to obtain a semisolid mass and vacuum dried to yield solid residues (5.24 % w/w chloroform extract and 6.22 % w/w ethanolic extract). The extracts were subjected to preliminary phytochemical investigation using standard methods [16,17]. The ethanolic extract of *Alstonia scholaris* was named as EEAS.

Chemicals and Reagents

α-tocopherol, nicotinamide adenine dinucleotide (NADH), butylated hydroxyanisole (BHA), butylated hydroxy toluene (BHT), l- ascorbic acid, nitroblue tetrazolium (NBT), phenazine methosulphate (PMS), the stable free radical 1,1-diphenyl-2-picryl-hydrazyl (DPPH), 3-(2-Pyridyl)-5,6-bis (4-phenyl-sulfonic acid)-1,2,4-triazine (Ferrozine), polyoxyethylenesorbitan monolaurate (Tween – 20) and trichloroacetic acid (TCA) were obtained from Sigma Aldrich, US. All other chemicals used were analytical grade and were obtained from Merck, US.

DPPH (1,1-Diphenly-2-picryl-hydrazil$^{\cdot}$) Free Radical Scavenging Activity:

The free radical scavenging activity of the ethanolic extract of *Alstonia scholaris* (EEAS) was measured by 1,1-diphenyl-2-picryl-hydrazil (DPPH$^{\cdot}$) using the method described by Shimada [18]. Briefly 0.1 mM solution of DPPH in ethanol was prepared; 1ml of the solution was added to 3 ml of EEAS in water at different concentrations (25-50 μg/ml). The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. Then the absorbance was measured at 517 nm by using a UV-Visible Spectrophotometer (Schimadzu UV-Vis 1700). Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The percent DPPH scavenging effect was calculated using the following equation:

DPPH$^{\cdot}$ scavenging effect (%) = $[(A_0-A_1)/A_0] \times 100$

where A_0 was the absorbance of the control reaction and A_1 was the absorbance in the presence of the standard sample or EEAS.

Ferrous Metal Ion Chelating Activity

The ferrous ions chelating by the EEAS and standards were estimated by the method of Dinis [19]. Briefly, the samples (25-50 μg/ml) were added to a solution of 2 mM $FeCl_2$ (0.05 ml). The reaction was initiated by the addition of 0.2 ml of 5 mM ferrozine and the mixture was shaken vigorously and left standing at room temperature for 10 min. After the mixture had reached equilibrium, the absorbance of the solution was measured spectrophotometrically at 562 nm by using a UV-Visible Spectrophotometer (Schimadzu UV-Vis 1700). All test and analyses were run in triplicate and averaged. The percentage of inhibition of ferrozine$^{\cdot}$-Fe^{2+} complex formation was found using the formula: % inhibition = $[(A_0-A_1)/A_0] \times 100$

where A_0 was the absorbance of the control reaction and A_1 was the absorbance in the presence of the standard sample or EEAS. The control contained $FeCl_2$ and ferrozine complex formation molecules.

Scavenging of Hydrogen Peroxide

The ability of EEAS to scavenge hydrogen peroxide was determined according to the method of Ruch [20]. A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4) and concentration was determined spectrophotometrically at 230 nm (Schimadzu UV-Vis 1700). EEAS (25-50 μg/ml) in distilled water was added to a hydrogen peroxide solution (0.6 ml, 40 mM) and the absorbance of hydrogen peroxide at 230 nm was determined after 19 min against a blank solution in phosphate buffer without hydrogen peroxide. The percentage of scavenging of hydrogen peroxide of EEAS and standard compounds was calculated using the following equation:

$$\% \text{ scavenged } [H_2O_2] = [(A_0-A_1)/A_0] \times 100$$

where A_0 was the absorbance of the control, and A_1 was the absorbance of EEAS or standards.

Superoxide Anion Radicals Scavenging Activity

Measurement of superoxide anion radicals scavenging activity of EEAS was based on the method described by Liu [21]. Superoxide radicals are generated in PMS – NADH systems by oxidation of NADH and assayed by the reduction of NBT. In these experiments, the superoxide radicals were generated in 3 ml of Tris-HCl buffer (16 mM, pH 8.0) containing 1 ml of NBT (50 μM), 1 ml NADH (78 μM) and EEAS (25 – 50 μg). The reaction was started by adding 1 ml of PMS solution (10 μM) to the mixture. The reaction mixture was incubated at 25° C for 5 min, the absorbance was read at 560 nm using a spectrophotometer (Schimadzu UV-Vis 1700) against blank samples using l- ascorbic acid as a control. Decreased absorbance of the reaction mixture indicated increasing superoxide anion scavenging activity. The percentage inhibition of superoxide anion generation was calculated using the following formula:

$$\% \text{ inhibition} = [(A_0-A_1)/A_0] \times 100$$

where A_0 was the absorbance of the control (l- ascorbic acid), and A_1 was the absorbance in the presence of EEAS or standards.

Total Reduction Capability

Total reduction capability of EEAS was estimated by using the method of Oyaizu [22]. EEAS (25-50 μg/ml) in 1 ml of distilled water was mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide [K_3Fe-$(CN)_6$] (2.5ml, 1%). The mixture was incubated at 50° C for 20 min by adding a 2.5 ml of 10 % trichloroacetic acid. Then the mixture was centrifuged for 10 min at 1000 x g. The upper layer of solu-

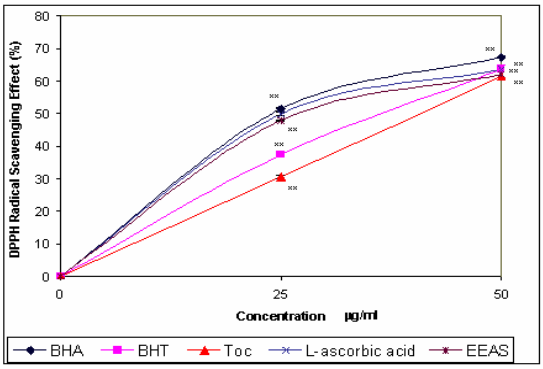

Fig 1. Free radical scavenging activity of different concentrations of standards and EEAS on DPPH radical.
ANOVA followed by Newmans-Keul multiple comparison test.
n =3, **p<0.01 compared to control

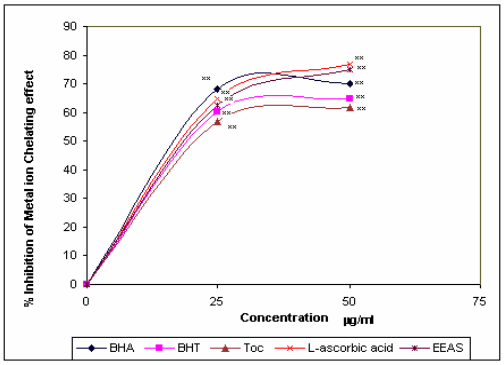

Fig 2. Ferrous Ions Chelating effect of Different Concentrations of EE ANOVA followed by Newmans-Keul multiple comparison test.
n =3, **p<0.01 compared to control
AS and standards.

tion (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%), and the absorbance was measured at 700 nm by a spectrophotometer (Schimadzu UV-Vis 1700). Higher absorbance of the reaction mixture indicated greater reducing power.

Statistical Analysis

Experimental results were mean ± SEM of three parallel measurements. Analysis of variance was performed by ANOVA followed by Newmans-Keul multiple comparison test. P values < 0.05 were regarded as significant.

RESULTS

Preliminary phytochemical investigation of the extracts showed the presence of alkaloids, tannins, flavonoids, saponins, glycosides and triterpenoids.

Free Radical Scavenging Activity by DPPH· Method:

A significant (p<0.01) decrease in the concentration of DPPH radical was observed due to the scavenging ability of the EEAS and standards (Fig 1). The standards used were BHA, BHT α-tocopherol and l- ascorbic acid. The scavenging effect of the EEAS and standards on the DPPH radical decreased in the order of BHA > l-ascorbic acid > EEAS> BHT >Toc and were 68, 63, 63, 62 and 61% at the concentration of 50 µg/ml, respectively, which were statistically significant (p<0.01) compared to the control. The effects of EEAS at 50 µg/ml reached a plateau as shown in Fig 1. Free radical scavenging activity increased with increasing concentration.

Ferrous Metal Ion Chelating Activity

The formation of Fe^{2+} -ferrozine complex is not completed in the presence of EEAS and standards which indicate that EEAS chelate the iron (Fig 2). The absorbance of Fe^{2+}-ferrozine complex was dose dependent and linearly decreased (from 25 to 50 µg/ml). The difference between EEAS and the control was statistically significant (p<0.01). The percentage of metal chelating capacity of 50 µg/ml concentration of EEAS, α-tocopherol, l-ascorbic acid, BHA and BHT were found to be 74.88, 61.92, 76.82, 70.02 and 64.62 %, respectively. The effects of EEAS reached a plateau in the 50 µg/ml concentration. The metal scavenging effect of EEAS and standards decreased in the order of l-ascorbic acid > EEAS > BHA > BHT > α-tocopherol.

Scavenging of Hydrogen Peroxide

25 µg/ml EEAS had strong H_2O_2 scavenging activity in comparison with the same doses of BHA, BHT, l-ascorbic acid and α-tocopherol. The percentage of H_2O_2 scavenging activity by same concentration (25 µg/ml) of EEAS, BHA, BHT, l-ascorbic acid and α-tocopherol were found to be 72.28, 83.65, 91.36, 72.56 and 90.1%, respectively, which were statistically significantly different (p<0.01) compared to the control (Fig 3). These results showed that EEAS had effective H_2O_2 scavenging activity and it was in the following order: BHT > α-tocopherol > BHA > l- ascorbic acid > EEAS.

Superoxide anion radical scavenging activity

Percentage inhibition of superoxide radical generation at 25 µg/ml concentration of EEAS was determined and compared with same doses of BHA, BHT, α-tocopherol and l- ascorbic acid. EEAS had strong superoxide radical scavenging activity comparable to that of BHA but lesser than BHT, α-tocopherol and l- ascorbic acid (Fig 4). The percentage inhibition of superoxide generation by 25 µg/ml concentration of EEAS was found to be 67.66 % whereas of BHT, α-tocopherol, l-ascorbic acid, BHA were found to be 81.56, 73.14, 70.21 and 68.83 %, respectively, which were statistically significant (p<0.01) from the control. Superoxide radical scavenging activity of these samples followed the order: BHT > α-tocopherol > l- ascorbic acid > BHA > EEAS.

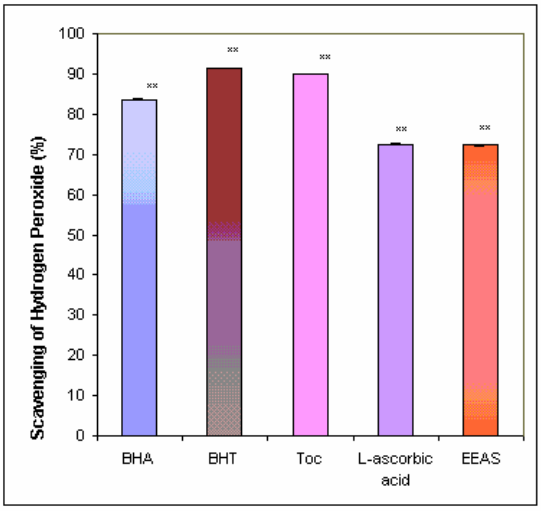

Fig 3. Scavenging of Hydrogen Peroxide of EEAS and standards
ANOVA followed by Newmans-Keul multiple comparison test.
n = 3, **p<0.01 compared to control.

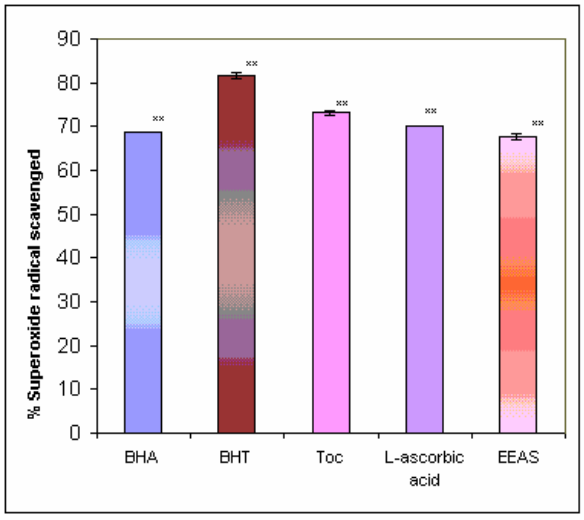

Fig 4. Superoxide anion radical scavenging activity of EEAS and standards
ANOVA followed by Newmans-Keul multiple comparison test

Total Reductive Capability by Potassium Ferricyanide Reduction Method

The reducing power of EEAS increased with increasing concentration (Fig 5). All the concentration of EEAS showed higher activities than BHT, α-tocopherol and l-ascorbic acid and these differences were statistically significant (p<0.05). EEAS and BHA had statistically similar reducing power (p>0.05). Reducing power of EEAS and standard compounds followed the order: BHA > EEAS > α-tocopherol > l-ascorbic acid > BHT respectively.

DISCUSSION

In our previous studies, the ethanolic extract of *Alstonia scholaris* was found to decrease the malondialdehyde level and prevented lipid peroxidation [10]. Reports suggested presence of nitric oxide scavenging activity in *Alstonia scholaris* [11]. But the *in vitro* antioxidant activity remained unexplored. Prompted by these findings it was decided to evaluate the antioxidant ability of ethanolic extract of *Alstonia scholaris* (EEAS) by *in vitro* antioxidant assays.

There are numerous antioxidant methods for evaluation of antioxidant activity. For *in vitro* antioxidant screening, ($DPPH^{\cdot}$) free radical scavenging, metal ion chelating, hydrogen peroxide scavenging, superoxide anion radical scavenging and Ferric thiocyanate reducing activities are most commonly used. However, the total antioxidant activity of an antioxidant cannot be evaluated by using one single method, due to oxidative processes. Therefore, at least two methods should be employed in order to evaluate the total antioxidant activity [23].

The model of scavenging the stable DPPH radical is a widely used method to evaluate antioxidant activities in a relatively short time compared with other methods. The effect of antioxidants on DPPH radical scavenging was thought to be due to their hydrogen donating ability. DPPH is a stable free radical and accepts an electron on hydrogen radical to become a stable diamagnetic molecule [24]. The decrease in absorbance of DPPH radical caused by antioxidants, because of the reaction between antioxidant molecule and radical progresses, results in the scavenging of the radical by hydrogen donation. It is visually noticeable as a discoloration from purple to yellow. Fig 1. indicates noticeable effect of EEAS on scavenging of free radicals. These results revealed that the EEAS is free radical inhibitor or scavenger acting possibly as primary antioxidants, which can be correlated with previous studies reported by Jeng-Leun Mau [25].

Iron can stimulate lipid peroxidation by the Fenton reaction and also accelerates peroxidation by decomposing lipid hydroperoxides into peroxyl and alkoxyl radicals that can themselves abstract hydrogen and perpetuate the chain reaction of lipid peroxidation [26]. In the presence of chelating agents, the complex formation is disrupted with the result that the red colour of the complex is decreased. Measurement of colour reduction therefore allows the estimation of the chelating activity of the coexisting chelator. In this assay, the EEAS and standard antioxidant compounds interfered with the formation of ferrous-ferrozine complex, suggesting that they had chelating activity and captured ferrous ion before ferrozine. The absorbance decreased linearly which indicated that the formation of Fe^{2+}-ferrozine complex was not completed in the presence of EEAS and the EEAS chelated the iron [27]. Metal chelating capacity was significant since it reduced the concentration of the

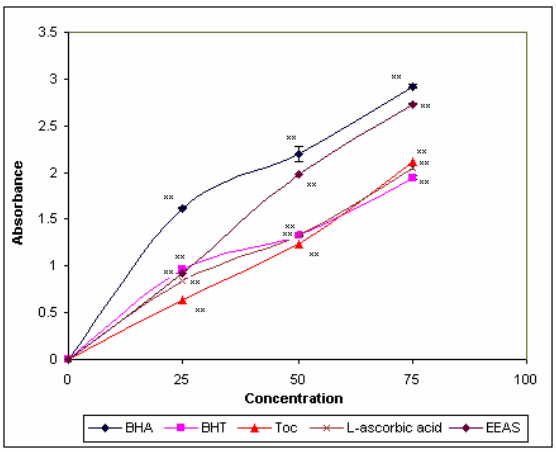

Fig 5. Total Reductive Capability of EEAS and standards. ANOVA followed by Newmans-Keul multiple comparison test.
n =3, ***p*<0.01 compared to control

catalyzing transition metal in lipid peroxidation. It was reported that chelating agents, which form σ bonds with a metal are effective as secondary antioxidants because they reduce the redox potential thereby stabilizing the oxidized form of the metal ion. The data obtained from Fig 2. reveals that EEAS demonstrated a marked capacity for iron binding, suggesting its action as peroxidation protector may be related to its iron binding capacity.

H_2O_2 is highly important because of its ability to penetrate biological membranes. H_2O_2 itself is not very reactive, but it can sometimes be toxic to cell because it may give rise to hydroxyl radical in the cells. The results showed that EEAS had an effective H_2O_2 scavenging activity.

Superoxide anion radicals are produced endogenously by flavoenzymes like xanthine oxidase, which converts hypoxanthine to xanthine and subsequently to uric acid in ischemia-reperfusion. Superoxide is generated *in vivo* by several oxidative enzymes, including xanthine oxidase. In the PMS-NADH-NBT system, superoxide anion derived from dissolved oxygen by PMS-NADH coupling reaction reduces NBT [23]. The decrease of absorbance at 560 nm with EEAS and antioxidants indicates the consumption of superoxide anion in the reaction mixture.

For the measurements of the reductive ability, the Fe^{3+}-Fe^{2+} transformation in the presence of EEAS was investigated and found to have significant reducing ability. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. The antioxidant activity of an antioxidant compound has been attributed to various mechanisms among which are prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging [23]. Generally flavonoids are the important class of antioxidants; hence the medicinal plants containing flavonoids and phenolic compounds are repeatedly screened for antioxidant activity.

In addition to flavonoids and phenolic compounds, some of the alkaloids, saponins and triterpenoids are reported to possess antioxidant activity. Eg. *Nelumbo nucifera* seeds [28]. The presence of flavonoids, alkaloids and triterpenoids in alcoholic extract of *Alstonia scholaris* has been reported [29] and the results of preliminary phytochemical investigation in the present study also further substantiates this. Hence, the observed *in vitro* antioxidant activity may be because of these phytoconstituents, which needs further investigation.

CONCLUSION

The results of the study clearly indicate that ethanolic extract of *Alstonia scholaris* (EEAS) possess powerful *in vitro* antioxidant activity. The encouraging results of EEAS with the various *in vitro* antioxidant tests proved the plant as a reducing agent, metal chelator, its hydrogen donating ability and effectiveness as scavengers of hydrogen peroxide, superoxide, and free radicals. Hence, it is worthwhile to isolate and elucidate the bioactive principles that are responsible for the antioxidant activity that is underway.

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REFERENCES

1. Versha P, Ghosh B, Anroop B, Ramanjit M. Antimicrobial activity of *Alstonia scholaris* leaf extracts. *Indian drugs*. 2003; 40: 412-413.
2. Nadkarni AK. Dr. KM Nadkarni's Indian Materia Medica, Vol. 1, Bombay, India. Popular Prakashan. 1976. p. 80-83.
3. Kirtikar KR, Basu BD. Indian Medicinal Plants, Vol.1, Allahabad, India. Lalit Mohan Basu. 2002. p. 111-14.
4. Keawpradub N, Kirby GC, Steele JCP, Houghton PJ. Antiplasmodial activity of extracts and alkaloids of three *Alstonia* species from Thailand. *Planta Medica*. 1999; 65: 690-694.
5. Lim-Sylianco CY, Jocano AP, Linn CM. Antimutagenicity of twenty Philippine plants using the micronucleus test in mice. *Philippine Journal of Science*. 1990; 117: 231-235.
6. Iwo MI, Soemardji AA, Retnoningrum DS, Sukrasno UUM. Immunostimulating effect of pule (*Alstonia scholaris* L. R.Br., Apocynaceae) bark extracts. *Clin Hemorheol Microcirc*. 2000J; 23:177-183.
7. Saraswathi V, Mathuram V, Subramanian S, Govindasamy S. Modulation of the impaired drug metabolism in sarcoma-180-bearing mice by echitamine chloride. *Cancer Biochem Biophys*. 1999; 17: 79-88.
8. Saraswathi V, Ramamoorthy N, Subramaniam S, Mathuram V, Gunasekaran P, Govindasamy S. Inhibition of glycolysis and respiration of sarcoma-180 cells by echitamine chloride. *Chemotherapy*. 1998; 44: 198-205.
9. Lin SC, Lin CC, Lin YH, Supriyatna S, Pan SL. The protective effect of *Alstonia scholaris R.Br.* on hepatotoxin-induced acute liver damage. *Am. J. Clin. Med.* 1996; 24: 153-164.

10. Arulmozhi.S, Rasal VP, Sathiyanarayanan L, Purnima Ashok. Screening of *Alstonia scholaris* Linn. R.Br., for wound healing activity. *Opem.* 2007; 7: 254-260.
11. Jagetia GC, Baliga MS. The evaluation of nitric oxide scavenging activity of certain Indian medicinal plants *in vitro*: a preliminary study. *J Med Food.* 2004; 7: 343 – 348.
12. Lorenz P, Roychowdhury S, Engelmann M, Wolf G, Horn TF. Oxyresveratrol and resveratrol are potent antioxidants and free radical scavengers: effect on nitrosative and oxidative stress derived from microglial cells. *Nitric Oxide.* 2003; 9: 64 – 76.
13. Es-Safi NE, Khlifi S, Kollmann A, El Abbouyi A, Ducrot PH. Antioxidant constituents of aerial parts of Globularia alypum growing in Morocco. *J Nat prod.* 2005; 68: 1293-6.
14. Couladis M, Tzakou O, Verykokidou E, Harvala C. Screening of same Greek aromatic plants for antioxidant activity. *Phytother Res.* 2003; 17:194-5.
15. Harish R. Shivanandappa. Antioxidant activity and hepatoprotective potential of *phyllanthus niruri*. *Food Chemistry*. 2006; 95: 180-185.
16. Trease GE, Evans WC. Pharmacognosy, 13th ed. Delhi, India. ELBS publication. 1989. p. 171 – 75.
17. Harborne JB. Phytochemical methods: A guide to modern techniques of plant analysis, 2nd ed. Newyork Chapman and Hall. 1984. p. 85 – 90.
18. Shimada K, Fujikawa K, Yahara K, Nakamura T. Antioxidative properties of xanthone on the auto oxidation of soybean in cylcodextrin emulsion. *J Agric Food Chem.* 1992; 40: 945 – 948.
19. Dinis TCP, Madeira VMC, Almeida LM. Action of phenolic derivatives (acetaminophen, salicylate, and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. *Arch Biochem Biophys.* 1994; 315: 161 – 169.
20. Ruch RJ, Cheng SJ, Klaunig JE. Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. *Carcinogenesis.* 1989; 10: 1003 – 1008.
21. Liu F, Ooi VE, Chang ST. Free radical scavenging activities of mushroom polysaccharide extracts. *Life Sci.* 1997; 60: 763-71.
22. Oyaizu M. Studies on products of browning reaction: Antioxidative activities of product6s of browning reaction prepared from glucosamine. *Jpn J Nutr.* 1986; 44: 307 – 315.
23. Ilhami Gulcin, Haci Ahmet Alici, Mehmet Cesur. Determination of *in vitro* antioxidant and radical scavenging activities of propofol. *Chem Pharm Bull.* 2005; 53: 281 – 285.
24. Soares JR, Dinis TCP, Cunha AP, Almeida LM. Antioxidant activities of some extracts of Thymus zygis. *Free Radical Research.* 1997; 26: 469 – 478.
25. Jeng-Leun Mau, Guei-Rung Chao and Kaun-Tzer Wu. Antioxidant properties of methanolic extracts from several ear mushrooms. *J Agric Food Chem.* 2001; 49: 5461-5467.
26. Halliwell B. Reactive oxygen species in living systems: source, biochemistry and role in human disease. *Am J Med.* 1991; 30: 14S – 22S.
27. Gulcin I, Buyukokuroglu ME, Oktay M, Kufrevioglu OI. Antioxidant and analgesic activities of turpentine of *Pinus nigra* Arn. subsp. pallsiana (Lamb.) Holmboe. *J Ethnopharmacol.* 2003; 86: 51-58.
28. Sujay Rai, Atul Wahile, Kakali Mukherjee, Bishnu pada Saha, pulok K. Mukherjee. Antioxidant activity of Nelumbo uncifera (sacred lotus) seeds. *J Ethnopharmacol.* 2006: 104: 322-27.
29. Khan MR, Omoloso AD, Kihara M. Antibacterial activity of *Alstonia scholaris* and *Leea tetramera*. *Fitoterapia.* 2003; 74: 736 – 40.

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